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A Comparison of the Daphnids *Ceriodaphnia dubia* and *Daphnia ambigua* for Their Utilization in Routine Toxicity Testing in the Southeastern United States

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Abstract. U.S. regulatory agencies commonly require effluent toxicity testing with *Ceriodaphnia dubia*—a practice that has led to the criticism that this species and test protocol often does not reflect local taxa or site-specific conditions. Using an indigenous test species may produce a more realistic model of local effects and may minimize test endpoint variance due to regional differences in water quality. This study addressed the substitution of *C. dubia* with *Daphnia ambigua* for toxicity testing in the southeastern United States. This investigation determined that *D. ambigua* could be laboratory cultured with only minimal changes to established regulatory protocol and that the life-cycle characteristics of this species were conducive to traditional acute and chronic aquatic toxicity test methods used with other daphnids. Acute toxicity tests showed that *D. ambigua* was less sensitive to some toxicants (sodium chloride, copper sulfate, and sodium lauryl sulfate) but more sensitive to others (chlorpyrifos). Chronic tests with copper sulfate and sodium chloride resulted in lower EC_{50}s for *D. ambigua* reproduction with both compounds. When exposed to low-alkalinity, low-pH stream waters typical of many southeastern United States watersheds, *C. dubia* demonstrated a significant reproductive depression in two of three streams tested, whereas *D. ambigua* experienced no chronic effect. These results suggest that *D. ambigua* may serve as a suitable surrogate for *C. dubia* as an toxicity indicator species in these types of receiving streams.

Short-term whole effluent toxicity testing generally follows several standard protocols (Lewis *et al.* 1994; ASTM 1993a, 1993b), which include a description of the use and culture of the common cladoceran species *Ceriodaphnia dubia. C. dubia* is favored by the U.S. Environmental Protection Agency (US EPA) because this species is easily cultured in the laboratory and extensive data are available on toxicant concentration-response relationships for this species. However, tests conducted with commonly used species do not always reflect local, site-specific conditions. For daphnid tests, the careful selection and use of indigenous species can account for regional differences in water quality—such as the very soft waters of the Southeastern United States, where *C. dubia* are not abundant—and may produce a more realistic test of local in-stream effluent effects.

The regulatory effluent discharge permits for the Savannah River Site (SRS), a U.S. Department of Energy facility located near Aiken, SC, requires toxicity testing using *C. dubia* at numerous outfalls on several receiving streams. However, water quality in these receiving streams, like most of those in the Southeastern United States, is markedly different from the standard laboratory water used for culturing and testing *C. dubia*. Local receiving stream alkalinities are very low ($<$20 mg/L as $CaCO_3$) and exhibit a relatively low pH. Furthermore, unimpacted receiving stream water from three on-site streams at SRS have even shown varying degrees of toxicity to this species (Specht 1994). Thus, it is possible that toxicity observed during chronic tests with *C. dubia* could be the result of dilution water alkalinity/pH dynamics and not the effects of contaminants in SRS effluents. Therefore, the objective of this study was to develop a daphnid toxicity test based on a local, site-specific daphnid species, *Daphnia ambigua*, which was presumed to be better adapted to the low alkalinity/low pH waters typical of the Southeastern United States.

D. ambigua was chosen as the most viable candidate for a site-specific routine test organism because it is commonly found in fresh waters of the Southeastern United States. It is also easy to culture in the laboratory. Hanazato (1990) has reported maintaining *D. ambigua* in laboratory cultures for more than 5 years, and La Point *et al.* (1995) found that *D. ambigua* can be cultured in very soft waters (12 mg $CaCO_3$/L). The objectives of our study were to investigate the following questions:

- Can *D. ambigua* be cultured in the laboratory under conditions that do not vary greatly from those already established by the US EPA, and are the life-cycle characteristics of this organism conducive for use with established toxicity testing protocols for *C. dubia*?

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- How sensitive is *D. ambigua* to four common toxicants compared to *C. dubia*?
- Will *D. ambigua* perform better than *C. dubia* in terms of control mortality, toxicant sensitivity, and reproduction in low alkalinity/low pH surface waters?

D. ambigua has been studied by various investigators for its tendency to produce a spike-like "helmet" on the anterior margin of the head when exposed to pesticides (Hanazato 1991, 1992, 1995) or certain predators (Hebert and Grewe 1985; Hanazato 1990 1991b 1991c, 1991d, 1995; Hanazato and Ooi 1992). Life history and energetics of *D. ambigua* have been studied by Lynch (1992), and a growth comparison of field and laboratory *D. ambigua* populations was conducted by Lei and Armitage (1980). There have been some toxicity studies using this organism (Leeper and Porter 1995; Hanazato 1991), and although none have addressed the use of *D. ambigua* as a replacement for the more common daphnid species used in regulatory testing, all have shown that laboratory culture of the organism is not difficult. Furthermore, La Point *et al.* (1995) showed that this species could be successfully cultured in the soft low-pH waters of the Southeastern United States, deeming it a possible replacement for *C. dubia* for site-specific regulatory toxicity testing.

Materials and Methods

D. ambigua *and* C. dubia *Cultures*

A collection of feral *D. ambigua* were netted from a pond in Aiken County, SC, and sorted from other zooplankton species. Approximately 50 individuals were pipetted into clean culture water and allowed to produce two to three broods over 9 days to establish stock cultures. Stocks were taxonomically verified from permanent mounts under phase-contrast microscopy following Pennak (1991) and Peters and De Bernardi (1987).

Laboratory cultures of *D. ambigua* were initiated and maintained for 16 months in 1.5-L culture dishes in moderately hard reconstituted water. Laboratory reconstituted water was prepared according to US EPA specifications (Lewis *et al.* 1994) using reagent-grade chemicals diluted in untreated well water that had been purified through a Nanopoure ultra-purification system (Barnstead Water Systems, Dubuque, IA). For the duration of this study, ranges in water quality parameters for the laboratory reconstituted water were as follows: hardness ranged from 54 to 72 mg/L as $CaCO_3$; alkalinity ranged from 56 to 76 mg/L as $CaCO_3$; and pH ranged from 8.2 to 8.5.

Culture dishes were kept in an environmental chamber with constant temperature (21 ± 2°C) and a photoperiod of 16:8 LD. Each culture dish contained 20–30 individual daphnids of the same age. Water in culture dishes was renewed at least three times per week. For *C. dubia*, a starter culture was obtained from a laboratory supplier and maintained in culture dishes as described with the exception of temperature (25 ± 2°C). A temperature of 25°C is critical if *C. dubia* are to produce three broods of young in 7 days—the time period required for routine regulatory testing (Lewis *et al.* 1994; McNaught and Mount 1985). *D. ambigua* cultures, however, were more robust when maintained at 21 ± 2°C, as recommended for other *Daphnia* species by the ASTM (1993b) and US EPA (Weber 1993).

Once a successful culture protocol and broodstock were developed, *D. ambigua* could be maintained in low-density cultures for a period of at least 7 weeks. In these cultures, 16 to 20 neonates of the same age were isolated into separate glass vials with 20 ml culture water. These individuals were maintained until they produced at least three broods of young. This approach was used to obtain baseline data on *D. ambigua* survival, reproduction, and culture-to-culture variability under laboratory conditions.

Both species of daphnids were fed a diet consisting of green algae (*Selenastrum capricornutum*) and a mixture of yeast, alfalfa, and fermented Trout Chow (Lewis *et al.* 1994). Four milliliters of an algal solution (7.0×10^7 cells/ml) and 4 ml of the yeast/alfalfa/Trout Chow mixture were added to each culture dish when the culture water was renewed (three times per week). Two milliliters of each food type were added on each day between water renewals. For low-density cultures, 100 μl/day of each food type was added to each vial.

Toxicity Testing

Toxicity testing was carried out in two distinct phases. The purpose of the initial phase (phase I) was to compare both the acute and chronic sensitivity of the two species when exposed to several toxicants in side-by-side testing scenarios. Phase I included:

- 48-h static acute toxicity tests using sodium chloride, copper sulfate, an organophosphorus insecticide (chlorpyrifos), and a detergent surfactant (sodium lauryl sulfate); and
- three-brood chronic toxicity tests with sodium chloride and copper sulfate.

The purpose of phase II was to gauge the performance of each species in bioassays of local stream water without the addition of a toxicant. Phase II measured whether unaltered surface water from three local receiving streams induced a toxic response in either species.

Phase I: Sensitivity Comparison: Static acute tests were performed according to test conditions and protocols described by the ASTM (1993b). Neonates of each species were exposed to a control treatment and an ascending series of five or six treatment concentrations prepared by spiking moderately hard reconstituted water with an appropriate volume of concentrated toxicant solution. Log order range-finding tests were conducted initially to aid in the selection of the final test concentrations. Five replicates, each containing 10 neonates, were prepared for each concentration. Replicates consisted of 250-ml glass beakers containing 100 ml test solution. Moderately hard reconstituted water served as the control solution and dilution water for these tests. Test vessels were randomly placed in environmental chambers under controlled photoperiod (16:8 LD) at 25 ± 2°C for *C. dubia* and 21 ± 2°C for *D. ambigua.* Dissolved oxygen concentrations ranged from 7.46 to 9.14 mg/L in test solutions, and pH ranged from 8.11 to 8.66.

A concentrated solution of chlorpyrifos was prepared by dissolving the compound in analytical-grade methanol at 40 mg/L. A concentrated working stock solution was then prepared by spiking dilution water with an aliquot of the methanol/chlorpyrifos mixture. The solvent was present in the highest test concentration at a level of 37.5 μl/L, a concentration well below the recommended 500 μl/L maximum (ASTM 1993b; US EPA 1985). A methanol solvent control was prepared at this concentration (37.5 μl/L) and included in each test. All other test compounds were dissolved directly into moderately hard reconstituted water to produce a concentrated spiking solution. Samples of all test solutions were preserved and analyzed, using the methodology to be described, to determine actual toxicant concentrations in test solutions.

Immobilization served as the endpoint for these toxicity tests, and results were expressed in terms of the 48-h LC_{50} (the toxicant concentration lethal to 50% of the test organisms). LC_{50}s were computed by the trimmed Spearman-Karber Method (Finney 1978) using the CT-TOX Multimethod Computer Program (CTDEP 1989) and measured toxicant concentrations. The LC_{50} values and their correspond-

ing 95% confidence limits served as an indicator of the relative sensitivities of these two species.

Chronic three-brood toxicity tests were conducted using sodium chloride and copper sulfate for comparison of the two species at sublethal levels. These tests were conducted following guidelines presented in ASTM (1993a) using moderately hard reconstituted water as a control and diluent. Neonates (<24 h old) of each species were exposed to a control treatment and an ascending series of treatment concentrations prepared by spiking dilution water with a concentrated solution of the toxicant. The selection of test concentrations was based on abbreviated range-finding tests. Twenty replicates, each containing one neonate, were prepared for each concentration; replicates consisted of 30-ml glass vials containing 20 ml test solution. Moderately hard reconstituted water served as the control solution and dilution water for these tests. Test solutions were renewed daily from stock solutions which were mixed on the first day of testing and stored at 4°C for the duration of the test. Test organisms were fed at a rate of 200 μl feeding solution/replicate/day. Toxicant and control solutions were renewed daily, but the duration of each chronic toxicity test depended on the species. Chronic toxicity tests were conducted until at least 60% of the control organisms had three broods of young. Typically, this took 7 days for *C. dubia* and 10 days for *D. ambigua.* Tests were conducted in environmental chambers under controlled photoperiod (16:8 LD) at 25 ± 2°C for *C. dubia* and 21 ± 2°C for *D. ambigua.* Dissolved oxygen concentration ranged from 7.45 to 9.27 mg/L, and pH ranged from 7.64 to 8.32 in test solutions.

To determine actual toxicant concentrations in each test, samples of renewal solutions were preserved for analysis. Mortality and reproduction served as test endpoints, and statistical analyses followed established guidelines (Lewis *et al.* 1994) for determining the no observed effect concentrations (NOEC) and lowest observed effect concentrations (LOEC). Depending on the distribution and variance of reproductive data for each toxicity test, statistical comparisons were made using either ANOVA and Dunnett's *t* test or Steel's many-one rank test. In addition to testing for statistical differences in mortality and reproduction, chronic test data were fitted to a nonlinear regression model, and the EC_{50} (median effective concentration) was extrapolated. Statistical analyses were performed using SAS and TOXSTAT (version 3.4) statistical software packages (SAS Institute 1987; Gulley 1994).

Test Compounds and Analytical Methods: Reagent-grade copper sulfate and sodium chloride were used in both acute and chronic toxicity testing. Copper and sodium in test solutions were measured using a Trace Scan ICP spectrophotometer (Thermo Jarrell Ash, model A-15). For the copper sulfate tests, results were reported in terms of total copper concentration. Measured sodium concentrations were used as a surrogate measure of sodium chloride in test solutions; results were reported as sodium chloride concentration. Sodium lauryl sulfate (reagent grade), used in acute tests, was analyzed as a methylene blue active substance following US EPA method 425.1 (US EPA 1983).

Research-grade chlorpyrifos (Dursban XP, DowElanco, Indianapolis, IN) was used in acute testing. Actual concentrations in test solutions were measured using a Perkin Elmer Q-Mass 910 benchtop GC/MS. Samples were prepared for analysis through a solid-phase microextraction technique (Webster *et al.* 1996), which used deuterated chlorpyrifos (CHPY-d10) as an internal standard and a polymer-coated fused silica fiber to extract the organic compounds from the analyte. The fiber, coated with polydimethylsiloxane and attached to a syringe-like apparatus, was exposed to the sample for approximately 15 min until the analyte had adsorbed to the polymer coating of the fiber. Once equilibrium was reached, the fiber was inserted into the injection port of the GC, where the analyte was thermally desorbed and the GC/MS procedure was completed using a selected ion-monitoring technique. Masses 314 and 324 were used for chlorpyrifos and CHPY-d10 quantitation, respectively. Temperature programming was 150°C for 1 min and 150 to 300°C at 20°C/min. Both the chlorpyrifos sample and the internal standard were baseline separated and eluted between 10.3 and 10.4 min. Analytical separations were carried out on a Perkin Elmer 1020 Autosystem gas chromatograph equipped with a 30-m DB-5 column with 0.25 mm inside diameter and 0.25 mm film thickness. Quantitation was performed using a Perkin Elmer Q-Mass 910 benchtop quadropole mass spectrometer.

Phase II: Species Comparison in Local Surface Waters: Because the primary purpose of this study was to evaluate the appropriateness of an indigenous test organism, it was valuable to evaluate the effects of local surface water on each of the two test species, both with and without the influence of a toxicant. Therefore, surface water was collected from three local streams on the SRS (Upper Three Runs, Pen Branch, and Fourmile Branch), at locations at least 1 km upstream from any outfall, and used for testing.

Initially, neonates of each species were exposed to water from each stream in a scenario similar to the one described for chronic toxicity testing. Twenty replicates, each containing one neonate, were prepared for each water type; replicates consisted of 30-ml containers filled with 20 ml test solution. Moderately hard reconstituted water served as the control solution and, because this was also the culture water, served as a basis of comparison for reproductive success. Solutions were renewed daily, and test organisms were fed at a rate of 200 μl feeding solution/replicate/day. These tests were continued until at least 60% of the control organisms had produced three broods of young. Mortality and reproduction served as endpoints for these tests. The statistical tests described in the preceding section were used to compare survival and reproduction of the control organisms (those in the laboratory culture water) to that of the test groups (those in the stream waters).

Results and Discussion

After some trial and error, it was determined that *D. ambigua* could be cultured using established regulatory *C. dubia* protocols (Lewis *et al*. 1994; Weber 1993) with two minor changes: a lower culture temperature (21°C) and a more dense feeding solution. Maintaining *D. ambigua* cultures at 25°C proved to be problematic in our laboratory due to recurring infections of ectoparasites in the cultures. A change to 21°C was chosen based on recommendations from the ASTM (1993b) and on reports of successful culture techniques from the literature (Leeper and Porter 1995). Another contributor to initial poor performance of *D. ambigua* in individual and mass cultures was insufficient food. When *D. ambigua* being fed an algal solution at the US EPA–recommended cell density (3.0 to 3.5×10^7 cells/ml) were compared to individuals being fed an algal solution with double that cell density, those with the higher-density diet were more robust and reproduction was in the range acceptable for toxicity testing (≥15 neonates in the first three broods). Mortality rates were high for those on the lower-density diet and reproduction was poor. Therefore, cell density in the algal feeding solution was increased to 7.0×10^7 cells/ml from the 3.0 to 3.5×10^7 cells/ml established by the US EPA.

Reproducing populations of *D. ambigua* were cultured for up to 16 months in our study. This culturability characteristic is particularly important when using organisms that are not commonly carried by commercial laboratory suppliers or that are unavailable from natural populations during certain times of the year. *D. ambigua* mass cultures also regularly produced adequate numbers of neonates for use in toxicity tests. When cultured individually, *D. ambigua* produced three broods of young within 10 to 11 days. Therefore, it could be used in

Table 1. Forty-eight-hour LC_{50} values for *D. ambigua* and *C. dubia* exposed to sodium chloride, sodium lauryl sulfate, copper, and chlorpyrifos

Toxicant	Exposure Concentration *C. dubia*	*C. dubia* # Dead	*C. dubia* # Alive	Exposure Concentration *D. ambigua*	*D. ambigua* # Dead	*D. ambigua* # Alive
Sodium chloride (g/L)	Control	0	50	Control	2	48
	0.479	1	49	0.479	1	49
	0.879	1	49	0.879	5	45
	1.27	4	46	1.27	8	42
	1.79	36	14	1.79	13	37
	2.26	50	0	2.26	38	12
	48-h LC_{50} (95% CL)	1.59 g/L (1.52–1.67 g/L)		48-h LC_{50} (95% CL)	2.00 g/L (1.81–2.20 g/L)	
Copper (μg/L)	Control	2	48	Control	1	49
	2.0	9	41	1.9	2	48
	3.05	10	40	2.9	2	48
	5.75	40	10	5.6	6	44
	8.35	49	1	8.5	49	1
	13.05	50	0	13.3	50	0
	22.45	50	0	21.8	50	0
	48-h LC_{50} (95% CL)	4.16 μg/L (3.70–4.69 μg/L)		48-h LC_{50} (95% CL)	6.53 μg/L (6.17–6.93 μg/L)	
Sodium lauryl sulfate (mg/L)	Control	0	50	Control	0	50
	0.953	13	37	0.953	0	50
	1.92	43	7	1.92	11	39
	2.90	50	0	2.90	33	17
	4.08	50	0	4.08	47	3
	6.15	50	0	6.15	49	1
	48-h LC_{50} (95% CL)	1.26 mg/L (1.14–1.39 mg/L)		48-h LC_{50} (95% CL)	44 mg/L (2.23–2.67 mg/L)	
Chlorpyrifos (μg/L)	Control	0	50	Control	1	49
	Solvent control	0	50	Solvent control	0	50
	0.03	0	50	0.02	0	50
	0.04	4	46	0.03	9	41
	0.07	47	3	0.04	39	11
	0.09	48	2	0.06	47	3
	0.19	50	0	0.08	50	0
	48-h LC_{50} (95% CL)	0.056 μg/L (0.054–0.059)		48-h LC_{50} (95% CL)	0.035 μg/L (0.032–0.037 μg/L)	

Values in parenthesis are 95% confidence limits.

standard three-brood toxicity tests with a 3 to 4 day extension of the test period established in accepted regulatory protocol (Lewis *et al.* 1994). Low-density cultures also showed a higher overall variance in *D. ambigua* reproductive data (compared to *C. dubia*), indicating that a higher number of replicates (20 as opposed to the recommended 10) would be a conservative recommendation for chronic toxicity testing.

Another overall disadvantage of *D. ambigua* was the organism's tendency to float in test solutions. This required careful handling during test solution renewal and was somewhat more time-consuming than working with *C. dubia*. Furthermore, this led to the use of glass test vessels as opposed to disposable plastic, which enhanced the tendency of floating organisms to stick to the sides.

Phase I: Species Sensitivity Comparison

Effluents generally contain a wide variety of toxicants, and test compounds were selected to reflect this variety. Although this study did not account for possible synergistic or antagonistic effects of these toxicants, as may be seen in an effluent mixture, it did provide a straightforward comparison for the expected effects of common toxicant classes on each test species.

In side-by-side acute tests with sodium chloride, copper sulfate, and sodium lauryl sulfate, LC_{50}s for *D. ambigua* tests were higher than those for *C. dubia* (Table 1). The opposite, however, was seen for the insecticide chlorpyrifos, for which the LC_{50} for *C. dubia* was somewhat higher than that for *D. ambigua* (0.056 μg/L and 0.035 μg/L, respectively). Because published *C. dubia* LC_{50} values for chlorpyrifos range from 0.058 to 0.079 μg/L (Bailey *et al.* 1996, 1997), it can be assumed that the 0.056 μg/L value obtained in this investigation was not unusually high. For each of the toxicants, the 95% confidence intervals for the two species did not overlap. These data indicate that acute sensitivity between the two species is similar within a factor of two, though significant differences were found for individual chemicals.

Chronic three-brood toxicity tests with sodium chloride and copper resulted in equivalent NOEC and LOEC values for both species. The NOEC/LOEC for both species exposed

Table 2. Results of chronic three-brood toxicity tests with *C. dubia* and *D. ambigua* exposed to sodium chloride

	Daphnia ambigua		*Ceriodaphnia dubia*	
Test Concentration (g NaCl/L)	Ending Survival (%)	Mean Number of Offspring per Female ±1 SD	Ending Survival (%)	Mean Number of Offspring per Female ±1 SD
Control	90	15.1 ± 4.6	100	23.6 ± 2.9
0.21	85	15.1 ± 10.3	100	22.0 ± 4.0
0.44	95	13.1 ± 3.4	100	21.8 ± 3.9
0.85	50[b]	3.1[b] ± 4.2	100	14.8[a] ± 4.8
1.3	30[b]	0.25[b] ± 0.8	95	12.3[a] ± 4.7
1.7	0[b]	0[b]	85	0.5[a] ± 0.9
2.2	10[b]	0[b]	0[b]	0[b]
EC_{50}	0.65 g NaCl/L (95% CI = 0.53–0.79)		1.35 g NaCl/L (95% CI = 1.12–1.64)	

Shaded rows represent the NOEC/LOEC concentrations based on Steel's many-one rank test, n = 20.
[a] Reproduction significantly reduced ($\alpha = 0.05$) when compared to the control group.
[b] Survival significantly reduced ($\alpha = 0.05$) when compared to the control group. These groups were not considered during the statistical comparison of reproduction.

Table 3. Results of chronic three-brood toxicity tests with *C. dubia* and *D. ambigua* exposed to copper sulfate

	Daphnia ambigua		*Ceriodaphnia dubia*	
Test Concentration (μg Cu/L)	Ending Survival (%)	Mean Number of Offspring per Female ± 1SD	Ending Survival (%)	Mean Number of Offspring per Female ±1 SD
Control	95	19.7 ± 5.9	100	28.4 ± 5.4
19	100	16.2 ± 5.3	95	26.2 ± 4.5
27	95	14.2[a] ± 6.1	90	23.5[b] ± 6.5
33	100	7.5[b] ± 5.0	60[b]	17.0[b] ± 9.4
39	65[b]	3.5[b] ± 3.5	50[b]	11.8[b] ± 8.0
49	55[b]	2.6[b] ± 4.0	65[b]	0.4[b] ± 1.2
53	45[b]	0.2[b] ± 0.6	100	2.4[a] ± 4.9
EC_{50}	30.4 μg Cu/L (95% CI = 28.6–32.3)		36.2 μg Cu/L (95% CI = 33.8–38.9)	

Shaded rows represent the NOEC/LOEC concentrations based on Steel's many-one rank test for *C. dubia* reproductive data and an ANOVA and Dunnett's *t* test for *D. ambigua* reproductive data; n = 20.
[a] Reproduction significantly reduced ($\alpha = 0.05$) when compared to the control group.
[b] Survival significantly reduced ($\alpha = 0.05$) when compared to the control group. These groups were not considered during the statistical comparison of reproduction.

to sodium chloride were 0.44 and 0.85 g NaCl/L, respectively (Table 2); the NOEC/LOEC values for copper were 19 and 27 μg Cu/L, respectively (Table 3). To gain more insight into the relative sensitivity of the two species, chronic test data was also used to calculate an EC_{50} for each species in each test. Unlike NOEC/LOEC values, which are dependent on the concentration series chosen for testing, the EC_{50} estimates a specific toxicant concentration at which 50% of the test population would be impacted. The EC_{50} for *C. dubia* in copper (36.2 μg Cu/L) was slightly higher than that of *D. ambigua* (30.4 μg Cu/L); but the *D. ambigua* EC_{50} from the sodium chloride test was half that of *C. dubia* (0.65 g NaCl/L versus 1.35 g NaCl/L). The 95% confidence intervals for the EC_{50}s did not overlap for either test (Tables 2 and 3). In both cases, sensitivities between the species were similar, indicating that *D. ambigua* may be no less protective than *C. dubia* for routine toxicity testing.

Phase II: Species Comparison in Local Waters

When exposed to the three local stream waters (Pen Branch, Four Mile Branch, and Upper Three Runs) in a chronic three-brood study, *C. dubia* showed a significant reduction in reproduction in two of the three streams tested when compared to reproductive rates in the laboratory control (Table 4). In contrast, *D. ambigua* reproductive rates increased in the stream waters when compared to the laboratory control group. Mortality was not a significant factor for either species during this phase of study.

These data demonstrated the reproductive success that could be expected from each species in local surface water and also showed that significant differences in reproductive output can arise merely from exposure to unaltered surface water. The depressed *C. dubia* reproductive response to stream waters could be attributed to differences in site-specific water quality

Table 4. Results of the daphnid three-brood stream-water reproductive success study performed with moderately hard reconstituted water and surface water from three local SRS streams (n = 20)

Water Type	Percent Survival		Mean Number of Young per Female ±1 SD	
	C. dubia	*D. ambigua*	*C. dubia*	*D. ambigua*
Control (reconstituted water)	100	100	32.2 ± 3.2	18.3 ± 4.2
Pen Branch	100	100	32.4 ± 2.5	24.8 ± 5.8
Four Mile Branch	100	100	9.8[a] ± 3.2	23.6 ± 5.4
Upper Three Runs	90	100	24.3[a] ± 5.5	24.5 ± 5.0

[a] Reproduction significantly reduced ($\alpha = 0.05$) when compared to the control group using Steel's many-one rank test.

Table 5. Summary of water quality parameters measured on daily renewal solutions for the SRS stream water daphnid reproduction study

Parameter	Water Type			
	Laboratory Reconstituted Water	Pen Branch	Four Mile Branch	Upper Three Runs
pH	8.22 (8.15–8.30)	7.64 (7.56–7.72)	5.63 (5.47–5.80)	6.53 (6.33–6.76)
Hardness (mg $CaCO_3$/L)	67.1 (58–72)	18.6 (16–22)	7.4 (6.0–8.0)	7.4 (6.0–10.0)
Alkalinity (mg $CaCO_3$/L)	59.2 (56–62)	25.4 (24.0–26.0)	5.5 (4.0–6.0)	3.8 (3.0–4.0)
Conductivity (μs/cm)	245.6 (239–251)	54.1 (53.1–54.7)	25.5 (24.9–25.7)	13.3 (13.1–13.5)
Dissolved oxygen (mg/L)	8.54 (8.34–8.75)	8.36 (8.24–8.67)	8.36 (8.24–8.54)	8.26 (8.16–8.42)

Values are expressed as means, ranges are presented in parentheses, n = 10.

versus that of traditional laboratory culture water (Table 5). Surface waters in Southeastern U.S. streams, such as Four Mile Branch and Upper Three Runs, are characterized by low alkalinity and pH, possibly accounting for the reduced performance of *C. dubia* when exposed to this water. Water from Pen Branch, which has a somewhat higher pH and hardness (Table 5), had no apparent negative effect on *C. dubia* during this 7-day period. Because moderately hard reconstituted water is the culture water recommended for *C. dubia* by the US EPA (Lewis *et al.* 1994), it is used in most U.S. commercial laboratories. The *C. dubia* reproductive effects demonstrated by this test are indicative of what may happen in actual regulatory tests that require the use of local surface water as a control and diluent. It is important that a test organism respond to added toxicants and not show decreased performance when exposed only to naturally occurring conditions. These results are consistent with those of Specht (1994), who found that *C. dubia* demonstrated impaired survival and/or reproduction when cultured in water from these same streams over a period of 11 months.

Conclusions

Our data suggest that *D. ambigua* would be a reasonable replacement for *C. dubia* for site-specific toxicity testing in low alkalinity/low pH receiving streams. It was determined that this species can be successfully cultured by basically following accepted guidelines for *C. dubia,* and that the life cycle characteristics of *D. ambigua* make it a viable candidate for routine aqueous toxicity testing. Disadvantages of this species, however, include some difficulty in handling and a greater reproductive variance over *C. dubia.* In both acute and chronic testing, the sensitivities of *D. ambigua* and *C. dubia* were comparable within a factor of two, with significant differences found for individual chemicals. The use of this resident species will present a more realistic organismal model for actual in-stream toxicant effects when low alkalinity (10–48 mg $CaCO_3$/L) and/or low pH are naturally occurring conditions. Also, when testing in receiving stream water, the use of *D. ambigua* may reduce the possibility of false-positive toxic effects produced by simple differences in dilution water alkalinity characteristics.

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